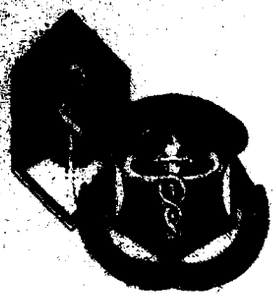


2

Institute Report No. 410

Acute Oral Toxicity of DIGL-RP Solid Propellant in ICR Mice

Larry D. Brown, DVM, LTC, VC
Conrad R. Wheeler, PhD
Yvonne C. LeTellier, BS
and
Don W. Korte, Jr., PhD, LTC, MSC

MAMMALIAN TOXICOLOGY BRANCH
DIVISION OF TOXICOLOGY

December 1989

Toxicology Series: 178

LETTERMAN ARMY INSTITUTE OF RESEARCH
PRESIDIO OF SAN FRANCISCO, CALIFORNIA 94129

Acute Oral Toxicity of DIGL-RP Solid Propellant in ICR Mice (Toxicology Series 178)--Brown *et al.*

This document has been approved for public release and sale; its distribution is unlimited.

Destroy this report when it is no longer needed. Do not return to the originator.

Citation of trade names in this report does not constitute an official endorsement or approval of the use of such items.

This research was conducted in compliance with the "Guide for the Care and Use of Laboratory Animals," NIH Publication No. 85-23, as prepared by the Institute of Laboratory Animal Resources, National Research Council.

This material has been reviewed by Letterman Army Institute of Research and there is no objection to its presentation and/or publication. The opinions or assertions contained herein are the private views of the author(s) and are not to be construed as official or as reflecting the views of the Department of the Army or the Department of Defense. *(AR 360-5)*

Donald G. Corby 8 Dec 89

Donald G. Corby (date)
COL, MC
Commanding

UNCLASSIFIED
SECURITY CLASSIFICATION OF THIS PAGE

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

1a. REPORT SECURITY CLASSIFICATION UNCLASSIFIED	1b. RESTRICTIVE MARKINGS
2a. SECURITY CLASSIFICATION AUTHORITY	3. DISTRIBUTION / AVAILABILITY OF REPORT APPROVED FOR PUBLIC RELEASE; DISTRIBUTION IS UNLIMITED.
2b. DECLASSIFICATION / DOWNGRADING SCHEDULE	
4. PERFORMING ORGANIZATION REPORT NUMBER(S) Institute Report No.: 410	5. MONITORING ORGANIZATION REPORT NUMBER(S)
6a. NAME OF PERFORMING ORGANIZATION Mammalian Toxicology Division of Toxicology 6b. OFFICE SYMBOL (If applicable) SGRD-ULE-T	7a. NAME OF MONITORING ORGANIZATION US Army Biomedical Research and Development Laboratory
6c. ADDRESS (City, State, and ZIP Code) Letterman Army Institute of Research Presidio of San Francisco, CA 94129-6800	7b. ADDRESS (City, State, and ZIP Code) Fort Detrick Frederick, MD 21701-5010
8a. NAME OF FUNDING / SPONSORING ORGANIZATION US Army Medical Research & Development Command 8b. OFFICE SYMBOL (If applicable)	9. PROCUREMENT INSTRUMENT IDENTIFICATION NUMBER
8c. ADDRESS (City, State, and ZIP Code) Fort Detrick Frederick, MD 21701-5012	10. SOURCE OF FUNDING NUMBERS PROGRAM ELEMENT NO: 62720 / PROJECT NO.: A835 / TASK NO.: AB / WORK UNIT ACCESSION NO.: DA303913
11. TITLE (Include Security Classification) (U) Acute Oral Toxicity of DIGL-RP Solid Propellant in ICR Mice	
12. PERSONAL AUTHOR(S) LD Brown, CR Wheeler, YC LeTellier, and DW Korte, Jr.	
13a. TYPE OF REPORT Institute	13b. TIME COVERED FROM 27FEB86 TO 3JUL86
14. DATE OF REPORT (Year, Month, Day) December 1989	15. PAGE COUNT 58
16. SUPPLEMENTARY NOTATION Toxicology Series No. 178	

17. COSATI CODES		
FIELD	GROUP	SUB-GROUP

18. SUBJECT TERMS (Continue on reverse if necessary and identify by block number)
DIGL-RP Solid Propellant, Diethyleneglycol Dinitrate, Mouse, Mammalian Toxicology, Propellants, Acute Oral Toxicity

19. ABSTRACT (Continue on reverse if necessary and identify by block number)

The acute oral toxicity of DIGL-RP solid propellant was determined in male and female ICR mice by using an oral gavage split-dose method. The MLD was 4176.1 ± 116.6 mg/kg for male mice and 3447.5 ± 42.1 mg/kg for female mice. DIGL-RP produced clinical signs that were attributed to its nitrate ester component, diethyleneglycol dinitrate. These signs included tremors, depression of reflexes, and inactivity. Other clinical signs observed were associated with the general malaise of the animals following dosing and included urine staining (males), hunched posture, squinting, and rough coat. Most animals exhibited signs by 2-3 hours after dosing and either had died or the signs had cleared by 96 hours after dosing. According to the classification scheme of Hodge and Sterner, these results place DIGL-RP in the slightly toxic class.

20. DISTRIBUTION / AVAILABILITY OF ABSTRACT ☒ UNCLASSIFIED/UNLIMITED ☐ SAME AS RPT ☐ DTIC USERS	21. ABSTRACT SECURITY CLASSIFICATION UNCLASSIFIED
22a. NAME OF RESPONSIBLE INDIVIDUAL DONALD G. CORBY, COL, MC	22b. TELEPHONE (Include Area Code) (415) 561-3600 22c. OFFICE SYMBOL SGRD-ULZ

DD Form 1473, JUN 86 *Previous editions are obsolete.*

SECURITY CLASSIFICATION OF THIS PAGE
UNCLASSIFIED

ABSTRACT

The acute oral toxicity of DIGL-RP solid propellant was determined in male and female ICR mice by using an oral gavage split-dose method. The MLD was 4176.1 ± 116.6 mg/kg for male mice and 3447.5 ± 42.1 mg/kg for female mice. DIGL-RP produced clinical signs that were attributed to its nitrate ester component, diethyleneglycol dinitrate. These signs included tremors, depression of reflexes, and inactivity. Other clinical signs observed were associated with the general malaise of the animals following dosing and included urine staining (males), hunched posture, squinting, and rough coat. Most animals exhibited signs by 2-3 hours after dosing and either had died or the signs had cleared by 96 hours after dosing. According to the classification scheme of Hodge and Sterner, these results place DIGL-RP in the slightly toxic class.

Key Words: Acute Oral Toxicity, DIGL-RP Solid Propellant, Diethyleneglycol Dinitrate, Mammalian Toxicology, Propellants, Mice

PREFACE

TYPE REPORT: Acute Oral Toxicity GLP Study Report

TESTING FACILITY:

US Army Medical Research and Development Command
Letterman Army Institute of Research
Presidio of San Francisco, CA 94129-6800

SPONSOR:

US Army Medical Research and Development Command
US Army Biomedical Research and Development Laboratory
Fort Detrick, MD 21701-5010
Project Officer: Gunda Reddy, PhD

PROJECT/WORK UNIT/APC: 3E162720A835/180/TLB0

GLP STUDY NUMBER: 85022

STUDY DIRECTOR: LTC Don W. Korte, Jr., PhD, MSC
Diplomate, American Board of Toxicology

PRINCIPAL INVESTIGATOR: LTC Larry D. Brown, DVM, VC, Diplomate
American Board of Toxicology, American
College of Veterinary Preventive Medicine

CO-PRINCIPAL INVESTIGATOR: Conrad R. Wheeler, PhD
Yvonne C. LeTellier, BS

PATHOLOGIST: MAJ Michael V. Slayter, DVM, VC

DATA MANAGER: Yvonne C. LeTellier, BS

REPORT AND DATA MANAGEMENT: A copy of the final report, study protocol, SOPs, raw data, analytical, stability, and purity data of the test compound, tissues, and an aliquot of the test compound will be retained in the LAIR Archives.

TEST SUBSTANCE: DIGL-RP Solid Propellant

INCLUSIVE STUDY DATES: 27 Feb 86 - 3 Jul 86

OBJECTIVE: The objective of this study was to determine the acute oral toxicity of DIGL-RP Solid Propellant in male and female ICR mice.

ACKNOWLEDGMENTS

SSG James D. Justus, BS, SP4 James J. Fischer, SP4 Scott L. Schwebe, SP4 Joel B. Seewald, BS, SP4 Dean K. Magnuson, BS, and SP4 Gayle A. Orner, BS, provided research assistance and animal care; SP4 John R.G. Ryabik, BS, and SP4 Paul B. Simboli, BS, provided chemical preparation and analysis; Richard A. Spieler, Charlotte L. Speckman, Obie Goodrich, SP4 Theresa L. Polk, and SP4 Gregory A. Rothhammer provided animal care and facility management; Colleen S. Kamiyama and Mara Joshua provided secretarial assistance.

SIGNATURES OF PRINCIPAL SCIENTISTS AND MANAGERS INVOLVED IN THE STUDY

We, the undersigned, declare that GLP Study 85022 was performed under our supervision, according to the procedures described herein, and that this report is an accurate record of the results obtained.

Don W. Korte, Jr. 21 Nov 89
DON W. KORTE, JR., PhD/DATE
LTC, MS
Study Director

Larry D. Brown 11 Jan 89
LARRY D. BROWN, DVM / DATE
LTC, VC
Principal Investigator

Yvonne C LeTellier 11/21/89
YVONNE C. LETELLIER, BS/DATE
DAC
Co-Principal Investigator/
Data Manager

Conrad Wheeler 21 Nov 89
CONRAD R. WHEELER, PhD/DATE
DAC
Co-Principal Investigator/
Analytical Chemist

DEPARTMENT OF THE ARMY

LETTERMAN ARMY INSTITUTE OF RESEARCH
PRESIDIO OF SAN FRANCISCO, CALIFORNIA 94129-6800

SGRD-ULZ-QA 30 November 1989

MEMORANDUM FOR RECORD

SUBJECT: GLP Compliance for GLP Study 85022

1. This is to certify that in relation to LAIR GLP Study 85022 the following inspections were made:

10 May 1985	-	Protocol Review
02 April 1986	-	Dosing

2. The institute report entitled "Acute Oral Toxicity (MLD) of DIGL-RP Solid Propellant in Mice," Toxicology Series 178, was audited on 17 August 1987.

Carolyn M Lewis

CAROLYN M. LEWIS
Diplomate, American Board of Toxicology
Quality Assurance Auditor

TABLE OF CONTENTS

Abstract.....i
Preface.....iii
Acknowledgments.....iv
Signatures of Principal Scientists.....v
Report of Quality Assurance Unit.....vi
Table of Contents.....vii

INTRODUCTION.....1

Objective of Study.....1

MATERIALS.....1

Test Substance.....1
Vehicle.....2
Animal Data.....2
Husbandry.....2

METHODS.....3

Group Assignment/Acclimation.....3
Dose Levels.....3
Compound Preparation.....4
Chemical Analysis of Dosing Suspensions.....4
Test Procedures.....4
Observations.....5
Necropsy.....5
Statistical Analysis.....5
Duration of Study.....5
Changes/Deviations.....5
Storage of Raw Data and Final Report.....6

RESULTS.....6

Mortality.....6
Lethal Dose Calculations.....6
Clinical Observations.....7
Pathology Findings.....10

DISCUSSION.....13

CONCLUSION.....13

REFERENCES.....14

TABLE OF CONTENTS (cont.)

APPENDICES.....15

Appendix A. Chemical Data.....16
Appendix B. Animal Data.....21
Appendix C. Historical Listing of Study Events.....22
Appendix D. Cumulative Mortality Data.....25
Appendix E. Individual Animal Histories.....26
Appendix F. Individual Body Weights.....43
Appendix G. Pathology Report.....53

OFFICIAL DISTRIBUTION LIST.....58

Acute Oral Toxicity of DIGL-RP Solid Propellant in ICR Mice--Brown *et al.*

INTRODUCTION

The Department of Defense is considering the use of diethyleneglycol dinitrate (DEGDN), triethyleneglycol dinitrate (TEGDN), or trimethylolethane trinitrate (TMETN) as a replacement for nitroglycerin in munition formulations. A "health effects" review conducted for the US Army Biomedical Research and Development Laboratory (USABDRL) identified numerous gaps in the toxicology database of these compounds (1). Consequently, USABDRL has tasked the Division of Toxicology, LAIR, to conduct an initial health effects evaluation of DEGDN, TMETN, TEGDN, and two DEGDN-based propellants, JA-2 and DIGL-RP. This initial evaluation includes the Ames mutagenicity assay, acute oral toxicity tests in rats and mice, acute dermal toxicity tests in rabbits, dermal and ocular irritation studies in rabbits, and dermal sensitization studies in guinea pigs.

Objective of Study

The objective of this study was to determine the acute oral toxicity of DIGL-RP Solid Propellant in male and female ICR mice.

MATERIALS

Test Substance

Chemical Name: DIGL-RP Solid Propellant

LAIR Code No.: TP57

Description: Solid black cylinders (stick configuration)

Lot Number: RAD83M001S169

DIGL-RP Solid Propellant was received in the stick configuration. It was ground into a fine powder for this study. Other test substance information is presented in Appendix A.

Vehicle

The vehicle for DIGL-RP was 1% gum tragacanth (Lot No. 34F0156, obtained from Sigma Chemical Company, St. Louis, MO) made up in sterile water for injection (Lot 65-914-DM-03, obtained from Abbott Laboratories, North Chicago, IL). The expiration date was Mar 1995 for the gum tragacanth and Jun 1995 for the sterile water for injection.

Animal Data

Seventy-nine male and 82 female ICR mice were obtained from Harlan Sprague-Dawley, Inc. (Indianapolis, IN) for this study. They were identified individually with cervical tags. Eighteen (9 of each sex) were used as approximate lethal dose (ALD) animals and two males and two females were submitted as necropsy quality controls. Thirty-nine females were removed from the study (see Changes/Deviations). Twenty-seven females were dosed with DIGL-RP and 5 were dosed with the vehicle. Fifty males were dosed with DIGL-RP and 5 were dosed with the vehicle. Unused male animals were transferred to another study. The animal weights on receipt ranged from 22 to 35 g. Additional animal data appear in Appendix B.

Husbandry

Mice were caged individually in stainless steel wire mesh cages in racks equipped with automatic flushing dumptanks. No bedding was used in any of the cages. The diet, fed *ad libitum*, consisted of Certified Purina Rodent Chow® Diet 5002 (Ralston Purina Company, St. Louis, MO); water was provided by continuous drip from a central line. The animal room temperature was maintained in a range from 21.1°C to 25.5°C with a relative humidity range of 38% to 60% with occasional spikes to 75% during room cleanings. The photoperiod was 12 hours of light per day.

METHODS

Group Assignment/Acclimation

Male and female mice were randomized separately into five dose groups and a vehicle control group with a stratified, weight-biased computer program (Beckman TOXSYS® Animal Allocation Program run on a Beckman TOXSYS® Data Collection Terminal). Due to the difficulties in initially administering the test compound to the female mice, the female mice dosed on the first day were removed from the study and the remaining females were reallocated among 4 dose groups (n=7) and a control (n=5). The animals were acclimated for 11-12 days before the day of dosing. During this period they were observed daily for signs of illness.

Dose Levels

The results of an approximate lethal dose (ALD) determination suggested that the median lethal dose (MLD) was approximately 3500 mg/kg. Based on these data, test doses were selected (Table 1).

TABLE 1: DIGL-RP Solid Propellant Doses

Male Dose Levels (mg/kg) target (actual)[a]	Female Dose Levels (mg/kg) target (actual)[a]
3160 (3532)[b,c]	
3500 (4122)[b]	2000 (2198)
3980 (4558)[b]	3160 (3092)
3500[c,d]	3500 (3858)
3980[d]	3980 (4454)
Vehicle	Vehicle

[a] Based on analysis of dosing suspensions.
[b] Initial dosing.
[c] Doses combined for data analyses using their mean for calculations.
[d] Suspensions were analyzed before dosing and dosing volumes were adjusted so that target concentrations were actually administered.

Compound Preparation

The DIGL-RP Solid Propellant (stick configuration) was ground into a fine powder before dosing using a Spex Model 6700 liquid nitrogen freezer/mill (Spex Industries, Inc, Edison, NJ). After passing through an 80-mesh sieve, this finely ground powder was weighed and mixed with appropriate volumes of a 1% solution of gum tragacanth to make dosing suspensions. Homogeneity was assured by mixing these suspensions with a Brinkman homogenizer. Because the homogenization process created numerous small air bubbles in the suspensions, they were prepared, placed overnight under refrigeration at 4°C, warmed and remixed gently the next morning, and then used.

Chemical Analyses of Dosing Suspensions

DIGL-RP was stable in the gum tragacanth vehicle for at least 24 hrs (Appendix A). This was sufficient since dosing was begun and completed within 20 hrs. Tests for homogeneity and concentration verification of the test compound in the gum tragacanth vehicle were conducted as outlined in Appendix A. The deviation of individual values from the mean of each set of 3 samples (top, middle, bottom) from each suspension did not exceed 3.1% for any suspension. The DIGL-RP dosing suspensions used in this study were within 97.8 - 118.2% of target. Since some concentrations deviated more than 10% from target, actual concentrations were used for data analyses or the dosing volume (Phase II males) was adjusted to obtain the targeted dose.

Test Procedures

This study was conducted in accordance with EPA guidelines (2) and LAIR SOP OP-STX-36 (3). The volume of dosing solution each animal received was based upon the desired dose level, the compound concentration in suspension, and the animal's weight. Dosing was performed using the oral gavage method without animal sedation or anesthesia. Since the test compound was viscous and thus difficult to administer at high concentrations, the animals were administered a split dose one hour apart to achieve the desired dose level. The dose level was increased by varying the concentration of each suspension. Split dose volumes ranged from 0.24 to 0.42 ml in the males and 0.27 to 0.36 ml in the females. The vehicle control (1% gum tragacanth) group received 0.31 to 0.32 ml (males) and 0.26 to 0.31 ml (females). The total volume administered each animal can be obtained by multiplying the split-dose volume by 2. The volumes given were based on a rate of 10 ml/kg for each split dose. Sterile disposable syringes (Monoject, Sherwood

Medical, St. Louis, MO) fitted with 20-21 gauge, 1.5-inch, ball-tipped feeding tubes (Popper & Sons, Inc., New Hyde Park, NY) were used. Dosing took place on six different days 4 hours after food had been removed from the animals' cages. Dosing began no soomer than 0932 hours and was concluded in all cases by 1240 hours (Appendix C).

Observations

Observations for mortality and signs of acute toxicity were performed daily according to the following procedure: (a) animals were observed undisturbed in their cages, (b) animals were removed from their cages and given a physical examination, and (c) animals were observed after being returned to their cages. On the day of dosing, the animals were checked intermittently throughout the day. Recorded observations were performed 2-3 and 4-5 hours after the initial dosing and daily for the remainder of the 2-week test period. A second "walk through" observation was performed daily with only significant observations recorded. Body weights were recorded once weekly during the course of the study.

Necropsy

Animals that died during the observation period were submitted for a complete gross necropsy. Those that survived the 14-day study period were submitted for necropsy immediately after receiving a barbiturate overdose.

Statistical Analysis

Statistical analyses were performed on the study results. The LD_{10}, LD_{50}, and LD_{90} were derived by probit analysis using the maximum likelihood method, as described by Finney (4). The program, PROBIT, developed for the Data General Computer, Model MV8000, was used to plot the probit curve and lethal dose values.

Duration of Study

Appendix C is a historical listing of study events.

Changes/Deviations

The dosing phase of this study was accomplished according to the protocol and applicable addenda with the following exceptions: The cage control group was not run as historical cage control data was available. Temperature and relative humidity fluctuated outside the specified ranges due to two power outages (29-30 May and 25 Jun 86). Due to the difficulty

inaccurately dosing the mice initially, the females dosed the first day were removed from the study and the remaining females were reallocated into 4 test groups containing 7 animals and a 5-animal vehicle control group. The second phase dosing of the males was accomplished after analysis of the dosing suspensions; therefore, the volumes administered were adjusted to obtain the targeted dose. Since the 3160 mg/kg and 3500 mg/kg dose groups actually received 3532 mg/kg and 3500 mg/kg these two groups were combined for data analyses. The DIGL-RP suspensions were administered as a split dose one hour apart because their high viscosity made concentrations greater than 200 mg/ml impossible to administer via the feeding tubes. Consequently, the first of 3 scheduled observations (1 hr after dosing) was deleted because the split-dosing procedure required 2 hrs instead of the normal 1 hr to complete. These deviations did not significantly affect the outcome of the study.

Storage of Raw Data and Final Report

A copy of the final report, study protocols, raw data, retired SOPs, and an aliquot of the test compound will be retained in the LAIR Archives.

RESULTS

Mortality

Thirty-one of 76 animals (17/49 males, 14/27 females) dosed with DIGL-RP died as a result of its toxicity. One (3.25%) deaths occurred within 24 hrs of dosing. An additional 20 (64.5%) deaths occurred by 48 hrs after dosing, and the remaining 10 (32.25%) deaths occurred within 96 hrs after dosing. Table 2 lists the compound-related deaths by dose group with percent mortality. Appendix D is a tabular presentation of the cumulative mortality data.

Lethal Dose Calculations

Lethal dose values were calculated by probit analysis and the equations for the probit regression line were: $Y = -63.0 + 18.8 \log X$ (males); $Y = -76.6 + 23.1 \log X$ (females), where X is the dose and Y the corresponding probit value. Animals removed from the study were not included in the calculations. Figures 1 and 2 graphically present the actual data points and the regression line. Lethal doses calculated from the equation for the probit regression line are presented in Table 3.

TABLE 2: Compound-Related Deaths by Group

Dose Level (mg/kg)	Deaths/ Group	Percent Mortality
	Males	
3516	1/20	5.0
3980	5/10	50.0
4122	4/9*	44.4
4558	7/10	70.0
Vehicle	0/5	0.0
	Females	
2196	0/6*	0.0
3092	1/7	14.3
3858	6/7	85.7
4454	7/7	100.0
Vehicle	0/5	0.0

* Reduced numbers in groups were due to animals which were misdosed and removed from the study.

TABLE 3: Calculated Lethal Doses (LD) of DIGL-RP Solid Propellant in ICR Mice

Level	Calculated Dose* (mg/kg)	95% Confidence Limits (mg/kg)
	Males	
LD_{10}	3568.9 ± 146.9	(3051.5, 3785.8)
LD_{50}	4176.1 ± 116.6	(3971.0, 4544.2)
LD_{90}	4886.6 ± 295.7	(4506.8, 6254.2)
	Females	
LD_{10}	3033.7 ± 90.3	(2234.3, 3311.8)
LD_{50}	3447.5 ± 42.1	(3063.2, 3802.5)
LD_{90}	3917.8 ± 220.8	(3605.6, 5085.2)

* Calculated dose ± standard error.

Clinical Observations

The most frequently observed category of clinical observations was behavioral disturbances (71 of 76 animals dosed). Behavioral signs exhibited by the animals included tremors, inactivity, twitching, irritability, hyperactivity,

Figure 1

DIGL-RP Dose Response Curve for Male Mice

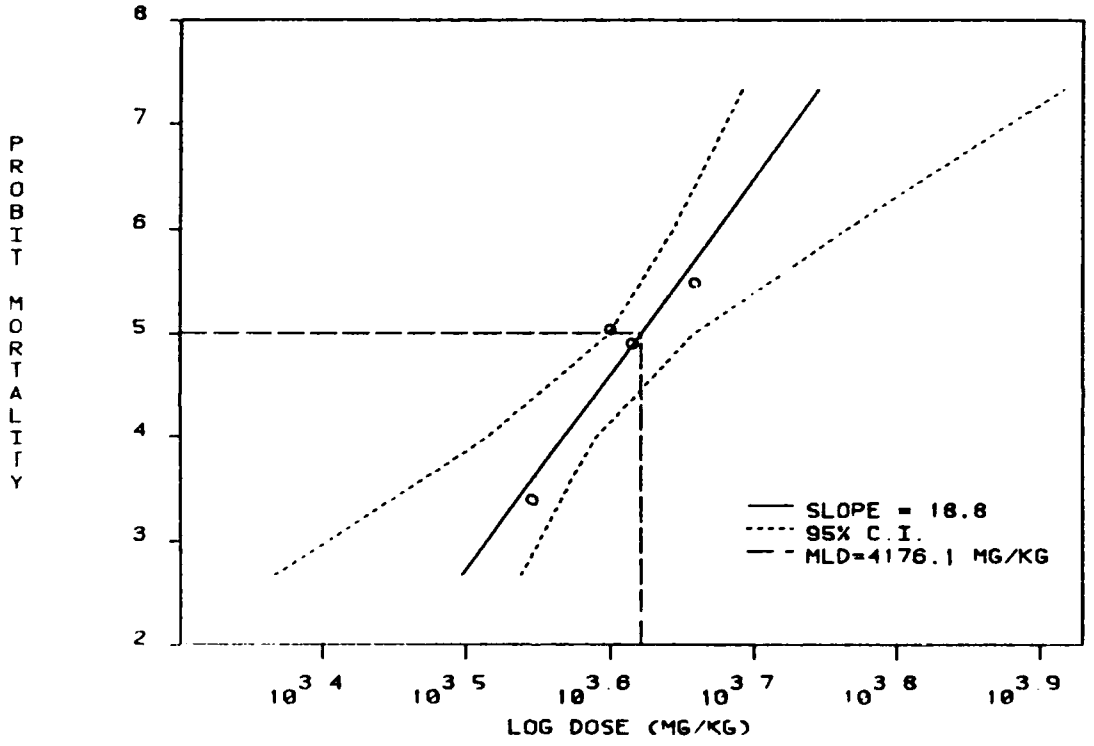
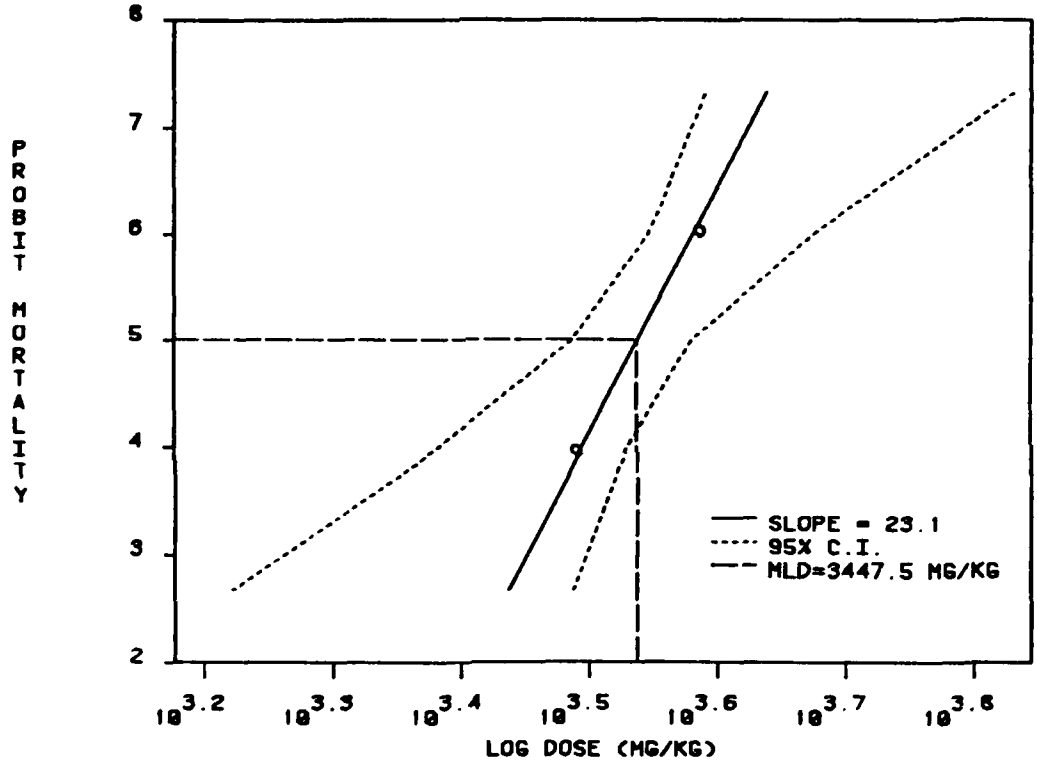


Figure 2

DIGL-RP Dose Response Curve for Female Mice



ataxia, and writhing. They were first observed 2 to 3 hrs after dosing and were generally no longer observed 48 hrs after dosing. All animals that died exhibited one or more behavioral signs. Other clinical signs attributed directly to the administration of DIGL-RP were changes in reflexes (23 of 76), changes in respiration (5 of 76), and opisthotonus (1 of 76).

Most other clinical signs were attributable to general malaise associated with the administration of DIGL-RP. Included with these signs were the categories of skin signs (rough coat) observed in 23 of 76, ocular signs (squinting) in 44 of 76, hunched posture in 64 of 76, and yellow (urine) stains in 22 of 76 (predominately males).

Three female and 4 male vehicle control animals were normal throughout the study. Signs observed in the vehicle control animals included hunched posture, hyperactivity, and rough coat and could be attributed to the dosing procedure and/or the volume of the gum tragacanth vehicle administered. Table 4 contains a summary of clinical observations. Appendix E contains individual animal histories.

Weight gains of survivors were not affected by administration of DIGL-RP. Table 5 presents the mean body weights by groups. Appendix F contains individual weight tables.

Pathology Findings

Gross and microscopic changes were noted only in animals that died during the study. With the exception of gastrointestinal hemorrhage, these changes were typical of those associated with postmortem events. The gastrointestinal hemorrhage was associated with males receiving the high dose.

TABLE 4: Incidence Summary for Clinical Observations in Mice Administered DIGL-RP Solid Propellant

MALES	Dose	Vehicle	3516	3980	4122	4558
	(n=)	5	20	10	9	10
Respiratory[a]		0	3	0	0	1
Behavorial[b]		1	19	8	9	10
Skin/Hair[c]		0	7	5	6	2
Gastrointestinal[d]		0	1	0	0	0
Stains[e]		0	12	2	5	2
Ocular[f]		0	10	6	6	10
Hunched posture		1	17	10	8	10
Reflex[g]		0	1	5	0	2
Prostrate/Moribund		0	0	3	1	3
Normal throughout		4	0	0	0	0
Deaths		0	1	5	4	7
FEMALES	Dose	Vehicle	2196	3092	3858	4454
	(n=)	5	6	7	7	7
Respiratory[a]		0	1	0	0	0
Behavorial[b]		1	4	7	7	7
Skin/Hair[c]		1	2	2	1	0
Opisthotonus		0	0	0	0	1
Stains[e]		0	0	0	1	0
Ocular[f]		0	0	0	6	6
Hunched posture		0	1	7	6	5
Reflex[g]		0	0	4	5	6
Prostrate/Moribund		0	0	2	1	6
Normal throughout		3	1	0	0	0
Deaths		0	0	1	6	7

[a] Includes increases in rate or depth.

[b] Includes tremors, inactivity, ataxia, hyperactivity, irritability, twitching, and writhing.

[c] Includes rough coat, pallor, and alopecia.

[d] Includes dehydration.

[e] Includes yellow (urine) stains on abdomen or perineum.

[f] Includes squinting.

[g] Includes changes in grasping, righting, and startle reflexes.

TABLE 5: Mean Body Weights in Grams ± S.E (N)

Dose Groups (mg/kg)	At Receipt	Dosing Day	Midtrial Day	Termination Day
		MALES		
3516	30.4 ±0.4 (20)	34.8 ±0.6 (20)	34.4 ±0.7 (19)	36.2 ±0.7 (19)
3980	28.6 ±0.5 (10)	32.2 ±1.2 (10)	30.2 ±1.6 (5)	32.8 ±1.6 (5)
4122	30.2 ±0.7 (9)	34.0 ±0.6 (9)	33.6 ±1.7 (4)	37.6 ±0.5 (4)
4558	29.2 ±0.7 (10)	33.2 ±0.7 (10)	33.3 ±0.7 (3)	37.0 ±2.0 (3)
Vehicle	24.8 ±0.6 (5)	30.6 ±0.4 (5)	33.2 ±0.9 (5)	34.2 ±0.8 (5)
		FEMALES		
2196	26.7 ±0.7 (6)	30.0 ±0.7 (6)	30.8 ±0.5 (6)	31.8 ±0.3 (6)
3092	25.7 ±0.7 (7)	31.1 ±0.6 (7)	31.7 ±0.8 (6)	31.7 ±0.5 (6)
3858	25.3 ±1.0 (7)	30.9 ±1.1 (7)	36.0 (1)	36.0 (1)
4454	25.6 ±0.7 (7)	31.1 ±0.6 (7)	N/A	N/A
Vehicle	25.0 ±0.9 (5)	29.0 ±0.7 (5)	30.0 ±0.9 (5)	30.8 ±0.8 (5)

DISCUSSION

The calculated median lethal dose (MLD) for DIGL-RP was 4176.1 mg/kg in male mice and 3447.5 mg/kg in female mice. These values place DIGL-RP within the slightly toxic classification (5).

DIGL-RP has as its major constituents, nitrocellulose and diethyleneglycol dinitrate (DEGDN) (Appendix A). Nitrocellulose is relatively nontoxic (MLD >5000 mg/kg) to mice (6) while a MLD of 1321-1395 mg/kg in mice has been reported (7). The spectrum of clinical signs (tremors, inactivity, depressed reflexes, etc.) observed following DIGL-RP administration supports the assumption that DEGDN (8) is the toxic component of DIGL-RP. The relative contribution of nitrocellulose and DEGDN to the MLD of DIGL-RP can be determined using their percentage compositions by weight (DIGL-RP is 62.5% nitrocellulose and 36.7% DEGDN). The calculated composition of DEGDN for the MLD of DIGL-RP is 1265-1533 mg/kg. These calculations reveal that the quantity of DEGDN present in DIGL-RP is roughly equivalent to its MLD while the quantity of nitrocellulose present is 40-50% of its MLD. Thus it is highly unlikely that nitrocellulose contributes significantly to the toxicity of DIGL-RP.

CONCLUSION

DIGL-RP Solid Propellant is a slightly toxic compound that produces tremors, inactivity, and depressed reflexes. Calculated MLD values were 4176.1 ± 116.6 mg/kg in male and 3447.5 ± 142.1 mg/kg in ICR mice.

REFERENCES

1. Holleman JW, Ross RH, Carroll JW. Problem definition study on the health effects of diethyleneglycol dinitrate, triethyleneglycol dinitrate, and trimethylolethane trinitrate and their respective combustion products. Frederick, MD: US Army Medical Bioengineering Research and Development Laboratory, 1983, DTIC NO. ADA 127846.

2. Enviromental Protection Agency. Office of Pesticides and Toxic Substances, Office of Toxic Substances (TS-792). Acute exposure, oral toxicity. In: Health effects test guidelines. Washington, DC; Environmental Protection Agency, August 1982; EPA 560/6-82-001.

3. Acute oral toxicity study (ALD and LD50). LAIR Standard Operating Procedure OP-STX-36, Presidio of San Francisco, California: Letterman Army Institute of Research, 15 June 1984.

4. Finney DJ. Probit analysis. 3rd ed. Cambridge: Cambridge University Press, 1971:20-80.

5. Hodge HC, Sterner JH. Tabulation of toxicity classes. Amer Ind Hyg Assoc Q 1943; 10:93-96.

6. Lee CC, Dilley JV, Hodgson JR, Helton DO, Wiegand WJ, Roberts DN, *et al.* Mammalian toxicity of munition compounds: Phase I. Acute oral toxicity, primary skin and eye irritation, dermal sensitization, and disposition and metabolism. Kansas City, MO: Midwest Research Institute, 1975, DTIC No. AD B011150.

7. Ryabik JRG, Brown LD, Wheeler CR, Korte DW, Jr. Acute oral toxicity of diethyleneglycol dinitrate (DEGDN) in mice. Toxicology Series 137. Presidio of San Francisco, CA: Letterman Army Institute of Research. Institute Report No. 336, May 1989.

8. Krasovsky GN, Korolev AA, Shigan SA. Toxicological and hygienic evaluation of diethyleneglycol dinitrate in connection with its standardization in water reservoirs. J. Hyg Epidemiol Microbiol Immunol 1973; 17:114-119.

Appendix A. Chemical Data.....16

Appendix B. Animal Data.....21

Appendix C. Historical Listing of Study Events.....22

Appendix D. Cumulative Mortality Data.....25

Appendix E. Individual Animal Histories.....26

Appendix F. Individual Body Weights.....43

Appendix G. Pathology Report.....53

Appendix A: CHEMICAL DATA

Chemical Name: DIGL-RP Solid Propellant

Physical State: Solid black cylinders (stick configuration)

Chemical analysis:

DEGDN was the only major component of DIGL which could be easily analyzed. For analysis, samples of DIGL powder were added to individual 100 ml volumetric flasks.[1] After dilution to volume with 90% ethanol, a second 1:100 dilution was performed. These solutions were analyzed by HPLC. Standards consisted of solutions of DEGDN in ethanol, ranging in concentration from 164.5 to 670.5 µg/ml. Analysis of DEGDN by HPLC was performed under the following conditions: column, Brownlee RP-18 (4.6 x 250 mm, Brownlee Labs, Inc., Santa Clara, CA); solvent system, 40% water - 60% acetonitrile); flow rate, 0.9 ml/min; wavelength monitored, 210 nm.[2] Under these conditions, DEGDN eluted with a retention time of approximately 5.4 min. The results from the analysis of standards and DIGL powder samples are presented in Tables 1 and 2.

Table 1. Analysis of Standards

Concentration of Standard (µg/ml)	Peak Area* (x 10^{-7})
164.5	0.94
191.0	1.09
275.5	1.60
299.4	1.74
362.0	2.08
399.6	2.31
444.4	2.52
539.8	3.07
585.0	3.32
670.5	3.79

*Average of 2 determinations

Equation for line by linear regression analysis:
$Y = 5.62 \times 10^4 X + 3.51 \times 10^5$, $r^2 = 0.9999$

[1] Wheeler CW. Toxicity testing of propellents. Laboratory Notebook #85-12-023, p. 51-61. Letterman Army Institute of Research, Presidio of San Francisco, CA.

[2] Wheeler CW. Nitrocellulose-nitroguanidine projects. Laboratory Notebook #84-05-010.3, p. 58. Letterman Army Institute of Research, Presidio of San Francisco, CA.

Appendix A (cont.): CHEMICAL DATA

Table 2. Analysis of DIGL Powder

Weight of DIGL Analyzed (mg)	Dilution Factor	Peak Area ($\times 10^{-7}$)	Conc. of DEGDN in DIGL (weight %)*
111.7	100	2.45	38.5
112.6	100	2.46	38.3
100.1	100	2.21	38.7

*Calculated using the following equation for the standard curve: = {[Peak Area - 3.51×10^5]/5.62×10^4} ÷ wgt DIGL (mg) × 10.

The average value for the concentration of DEGDN in DIGL was 38.5% and this agrees closely with the value of 36.70 ± 1.50 reported in the manufacturer's data sheet.

Preparation of test substance: The cylinders of DIGL were ground under liquid nitrogen using a Spex freezer mill. After grinding, the powder was sieved through an 80-mesh screen.

Stability:

The aqueous stability of the DEGDN component in the DIGL powder was examined.[3] The amount of DEGDN in aqueous DIGL suspensions was determined immediately after preparation of a suspension and again 24 hrs later. The study was conducted as follows. A suspension of DIGL in 1% gum tragacanth (200 mg/ml) was prepared. Three 1 ml aliquots were removed from the suspension immediately after preparation and again 24 hrs later. The 1 ml samples were transferred to individual 100 ml volumetric flasks. After diluting to volume with ethanol, the flasks were shaken well. A sample from each was analyzed by HPLC as described above. The average of the peak area values was 4.03 ± 0.12 for the 0 time samples and 4.10 ± 0.14 for the 24-hour samples. These results indicate that there was no decomposition of DEGDN in 1% gum tragacanth for a period of 24 hours.

Source: Radford Army Ammunition Plant, Radford, Virginia (prime contractor: Hercules, Inc., Wilmington, DE)

LAIR Code Number: TP57

Lot No.: RAD83M001S169

[3] Wheeler CW. Toxicity Testing of Propellents. Laboratory Notebook #85-12-023, p. 24-42. Letterman Army Institute of Research, Presidio of San Francisco, CA.

Appendix A (cont.): CHEMICAL DATA

Homogeneity[4]: Suspensions (20 ml) of DIGL-RP powder were prepared in 1% gum tragacanth at concentrations of approximately 50, 100 and 200 mg/ml. After withdrawing one ml from the top, middle, and bottom of each suspension and diluting with ethanol the samples were analyzed by HPLC for DEGDN content. The suspensions were homogeneous since no individual value deviated more than 10% from the mean value for each concentration tested.

Table 3. Analysis of DEGDN Standards

Concentration of DEGDN (µg/ml)	Peak Area* (x 10^6)
76.9	4.46
95.8	5.59
158.2	9.18
195.0	11.26
279.6	16.21
306.9	17.75
340.2	19.50
413.1	23.70

*Average of standards run before and after samples.
Equation for line by linear regression analysis: $Y = 5.7 \times 10^4 X - 1.1 \times 10^5$, $r^2 = 0.999$

Table 4. Analysis of DIGL Samples for Homogeneity

Concentration (mg/ml)	Dilution Factor (D.F.)	Peak Area x 10^6	Conc. of DIGL* (mg/ml)
50.6 T	100	11.39	51.5
50.6 M	100	11.21	50.6
50.6 B	100	11.76	53.1
100.5 T	250	8.90	100.2
100.5 M	250	9.02	101.6
100.5 B	250	8.85	99.7
198.9 T	500	8.12	182.7
198.9 M	500	7.99	179.7
198.9 B	500	8.26	185.9

*Conc of DIGL-RP (mg/kg) = [(peak area - 1.1×10^5)/5.7×10^4] x D.F. x 2.60/1000 µg/mg

[4] Wheeler CW. Toxicity testing of propellents. Laboratory Notebook #85-12-023.1, p. 2-5. Letterman Army Institute of Research, Presidio of San Francisco, CA.

Appendix A (cont.): CHEMICAL DATA

Analysis of Dosing Suspensions[5,6]: DIGL dosing suspensions were analyzed by HPLC to determine concentration accuracy. As with the homogeneity determinations, this was accomplished by anaylzing for the DEGDN component of DIGL. Since seven of nine dosing suspensions differed from the target concentration by greater than 10% (Table 5), the actual concentration should be used for data analyses.

Table 5. Concentration of DIGL in Dosing Suspensions

Target Conc. (mg/ml)	Date Prepared	Date Analyzed	Dilution Factor	Peak Area	Conc. of DIGL (mg/ml)	% Target Conc.
			FEMALES			
100.0	18/3/86	27/3/86[a]	100	4751	109.8	109.8
158.0	25/3/86	27/3/86[a]	100	6605	154.6	97.8
175.0	8/4/86	14/4/86[b]	200	4147	192.9	110.2
199.0	1/4/86	14/4/86[b]	500	2034	222.7	111.9
			MALES			
158.0	9/6/86	11/6/86[c]	100	7554	176.6	111.8
175.0	9/6/86	11/6/86[c]	200	4505	206.1	117.8
175.0	17/6/86	18/6/86[d]	200	4305	206.9	118.2
199.0	9/6/86	11/6/86[c]	200	4956	227.9	114.5
199.0	17/6/86	18/6/86[d]	200	4716	227.7	114.4

Equations for the standard curves and the %DEGDN in DIGL-RP:

[a] Y (peak area) = 10.52 X (µg/ml) + 213.9, R^2 = 0.999. %DEGDN = 39.3

[b] Y (peak area) = 10.75 X (µg/ml) + 220.5, R^2 = 0.999. %DEGDN = 37.9

[c] Y (peak area) = 10.63 X (µg/ml) + 232.3, R^2 = 0.999. %DEGDN = 39.0

[d] Y (peak area) = 10.65 X (µg/ml) + 224.9, R^2 = 0.999. %DEGDN = 37.0

[5] Wheeler CR. Toxicity testing of propellants. Laboratory Notebook #85-12-023.1, p. 30-44, 69-75. Letterman Army Institute of Research, Presidio of San Francisco, CA.

[6] Wheeler CR. Toxicity testing of propellants. Laboratory Notebook #85-12-023.2, p. 1-10. Letterman Army Institute of Research, Presidio of San Francisco, CA.

Appendix A (cont.): CHEMICAL DATA

Manufacturer's Data Sheet for DIGL-RP Formulation

Finished Propellant Ingredients	Percentage
Nitrocellulose (13.05 ±0.05% Nitrogen) (6-12 seconds viscosity)	62.5 ±2.00
Diethyleneglycol Dinitrate (DEGDN)	36.70 ±1.50
Ethyl Centralite (EC)	0.25 0.25 ±0.05
Akardit II	0.25 0.45 ±0.15
Magnesium Oxide	0.05 Max
Graphite (Chg 5)	0.05 Max
	100.00

Appendix B: ANIMAL DATA

Species: Mus musculus

Strain: Albino ICR (Institute of Cancer Research)

Source: Harlan Sprague-Dawley, Inc.
Indianapolis, IN

Date of Arrival: Male controls and females on 27 February 86
Treated males on 28 May 86

Sex: Male and female

Date of Birth: DIGL-RP Males - 4 Apr 1986
Vehicle Males - 17 Jan 1986
Females - 3 Jan 1986

Method of Randomization: Weight bias, stratified animal allocation.

Initial Animal Allocation: 10/group, male or female, except vehicle groups had 5 each

Animal Condition at Study Initiation: Normal

Body Weight Range at Dosing: 27 - 40 g

Identification Procedures: Cervical tag.

Conditioning: Quarantine/acclimation 28 Feb - 10 Mar 86 for females and male vehicle controls and 29 May - 9 Jun 86 (12 days) for males.

Justification: The laboratory mouse has proven to be a sensitive and reliable animal model for lethal dose determinations.

Appendix C: HISTORICAL LISTING OF STUDY EVENTS

FEMALES AND MALE VEHICLE CONTROLS

Date	Event
27 Feb 86	Received ICR mice. Animals were checked for physical condition, sexed, tagged, weighed, and individually caged.
28 Feb 86	Mice were submitted for necropsy quality control.
28 Feb - 10 Mar 86	Animals were observed daily.
3 Mar 86	Animals were weighed and randomized into dose groups.
10 Mar 86	Animals were weighed and removed from quarantine.
4-7 Mar 86	ALD animals were dosed, and observed.
19 Mar 86	Phase I animals (2196 mg/kg females and controls and male controls) were weighed, dosed, and observed at 2-3 and 4-5 hours after dosing.
20 Mar - 2 Apr 86	Phase I animals were observed daily in the am and pm.
25 Mar 86	Phase I animals were weighed.
26 Mar 86	Phase II animals (3092 mg/kg females) were weighed, dosed, and observed at 2-3 and 4-5 hours after dosing.
27 Mar - 9 Apr 86	Phase II animals were observed daily in the am and pm.
2 Apr 86	Phase I animals were weighed, observed and submitted to necropsy. Phase II animals were weighed. Phase III animals (4454 mg/kg females) were weighed, dosed, and observed at 2-3 and 4-5 hours.
3 - 6 Apr 86	Phase III animals were observed daily in the am and pm.

Appendix C (cont.): HISTORICAL LISTING OF STUDY EVENTS

FEMALES AND MALE VEHICLE CONTROLS

Date	Event
9 Apr 86	Phase II animals were weighed, observed, and submitted to necropsy. Phase IV animals (3858 mg/kg females) were weighed, dosed and observed at 2-3 and 4-5 hours.
10 - 23 Apr 86	Phase IV animals were observed daily in the am and pm.
16 Apr 86	Phase IV animals were weighed.
23 Apr 86	Phase IV animals were weighed, observed, and submitted for necropsy.

Appendix C (cont.): HISTORICAL LISTING OF STUDY EVENTS

TREATED MALES

Date	Event
28 May 86	Received ICR mice. Mice were checked for physical condition, sexed, weighed, tagged, and individually caged.
29 May 86	Mice were submitted for necropsy quality control.
29 May - 9 Jun 86	Animals were observed daily.
4 Jun 86	Animals were weighed.
5 Jun 86	Animals were randomized into dose groups, and removed from quarantine.
10 Jun 86	Phase I animals (3532, 4130, and 4556 mg/kg) were weighed, dosed, and observed at 2-3 and 4-5 hours after dosing.
11 - 24 Jun 86	Phase I animals were observed daily in the am and pm.
17 Jun 86	Animals were weighed.
19 Jun 86	Phase II animals (4130 and 4556 mg/kg) were weighed, dosed and observed at 2-3 and 4-5 hours after dosing.
20 Jun -3 Jul 86	Phase II animals were observed daily in the am and pm.
24 Jun 86	Animals were weighed. Phase I animals were observed and submitted to necropsy.
1 Jul 86	Phase II animals were weighed.
3 Jul 86	Phase II animals were weighed, observed, and submitted to necropsy.

Appendix D: CUMULATIVE MORTALITY DATA (deaths/group)

Dose mg/kg	Animals/ Group	Time After Dosing: Hours 2	Hours 4	Days 1	2	3	4	5	6	7	8-14
				MALES							
3516	20	0	0	0	1	1	1	1	1	1	1
3980	10	0	0	0	2	4	5	5	5	5	5
4122	9	0	0	0	3	4	4	4	4	4	4
4558	10	0	0	0	5	7	7	7	7	7	7
Vehicle	5	0	0	0	0	0	0	0	0	0	0
TOTAL*	49	0	0	0	11	16	17	17	17	17	17
				FEMALES							
2196	6	0	0	0	0	0	0	0	0	0	0
3092	7	0	0	1	1	1	1	1	1	1	1
3858	7	0	0	0	3	6	6	6	6	6	6
4454	7	0	0	0	6	7	7	7	7	7	7
Vehicle	5	0	0	0	0	0	0	0	0	0	0
TOTAL*	27	0	0	1	10	14	14	14	14	14	14

* TOTAL reflects only animals receiving DIGL-RP.

APPENDIX E: INDIVIDUAL ANIMAL HISTORIES

MALE: 3516 mg/kg DIGL-RP

Animal Number	Clinical Signs	Dates Observed (1986)	Severity
86C00176	Hunched Posture	June 10,11	Slight
	Tremors	June 10	Slight
	Inactive	June 10,11	Slight
	Ataxia	June 10	Slight
86C00177	Inactive	June 19,20	Moderate
	Incr. Respiratory Depth	June 19	Moderate
	Hunched Posture	June 19,20	Moderate
	Twitching	June 19	Slight
	Tremors	June 19,20	Moderate
	Depr. Righting Reflex	June 19	Slight
	Squinting	June 19,20	Moderate
	Stain, Perianal, Yellow	June 20	Slight
	Death	June 21	1.9 days
86C00182	Squinting	June 10	Slight
	Hunched Posture	June 10,11	Slight
	Ataxia	June 10,11	Slight
	Tremors	June 10	Slight
	Stain, Abdomen, Yellow	June 10,11	Slight
86C00183	Hunched Posture	June 19,20	Slight
	Inactive	June 19	Slight
	Ataxia	June 19,20	Slight
	Tremors	June 19,20	Slight
	Rough Coat	June 19-21,23	Slight
	Stain, Perianal, Yellow	June 20,21,23	Moderate
86C00187	Inactive	June 19	Slight
	Hunched Posture	June 19,20	Slight
	Tremors	June 19,20	Moderate
	Rough Coat	June 19-21	Slight
	Ataxia	June 19	Slight
	Stain, Abdomen, Yellow	June 20	Slight
86C00190	Hunched Posture	June 19	Slight
	Ataxia	June 19	Moderate
	Inactive	June 19	Moderate
	Tremors	June 19	Slight
	Rough Coat	June 19	Slight
	Stain, Abdomen, Yellow	June 19,20	Moderate

APPENDIX E (cont.): INDIVIDUAL ANIMAL HISTORIES

MALE: 3516 mg/kg DIGL-RP (cont.)

Animal Number	Clinical Signs	Dates Observed (1986)	Severity
86C00196	Hunched Posture	June 19	Slight
	Stain, Perianal, Yellow	June 19,20	Slight
86C00202	Inactive	June 10	Slight
	Hunched Posture	June 10,11	Slight
	Ataxia	June 10	Slight
	Tremors	June 10	Slight
	Stain, Abdomen, Yellow	June 10	Moderate
86C00204	Hunched Posture	June 19,20	Slight
	Incr. Respiratory Rate	June 19	Slight
	Inactive	June 19,20	Slight
	Tremors	June 19,20	Slight
	Ataxia	June 19	Slight
86C00205	Hunched Posture	June 19	Slight
	Inactive	June 19	Slight
86C00208	Inactive	June 10,11	Marked
	Squinting	June 10,11	Marked
	Rough Coat	June 10,11	Moderate
	Tremors	June 11	Slight
86C00213	Inactive	June 10,11	Marked
	Tremors	June 10,11	Moderate
	Stain, Abdomen, Yellow	June 10-12	Moderate
	Squinting	June 10,11	Moderate
	Hunched Posture	June 12,13	Marked
	Stain, Perianal, Yellow	June 15,16,19,20	Slight
	Hyperactive	June 20	Moderate
86C00215	Hunched Posture	June 19,20	Moderate
	Incr. Respiratory Rate	June 19	Moderate
	Tremors	June 19	Slight
	Hyperactive	June 20	Slight
86C00220	Inactive	Jun 10,11	Marked
	Squinting	June 10	Slight

APPENDIX E (cont.): INDIVIDUAL ANIMAL HISTORIES

MALE: 3532 mg/kg DIGL-RP (cont.)

Animal Number	Clinical Signs	Dates Observed (1986)	Severity
86C00224	Inactive	June 10,11	Moderate
	Hunched Posture	June 10,11	Moderate
	Squinting	June 10,11	Slight
	Stain, Abdomen, Yellow	June 10,11	Moderate
	Stain, Perianal, Yellow	June 14	Slight
86C00226	Hunched Posture	June 19,20	Marked
	Tremors	June 19,20	Moderate
	Inactive	June 19	Moderate
	Ataxia	June 19,20	Slight
	Squinting	June 19	Moderate
86C00233	Hunched Posture	June 10	Moderate
	Inactive	June 10-12	Marked
	Tremors	June 10,12	Slight
	Ataxia	June 10,11	Moderate
	Squinting	June 10,11	Marked
86C00237	Tremors	June 19	Slight
	Hunched Posture	June 19-21	Slight
	Rough Coat	June 19,20	Slight
	Stain, Perianal, Yellow	June 20	Slight
86C00239	Inactive	June 10	Marked
	Hunched Posture	June 10,11	Moderate
	Rough Coat	June 10,11,15-17	Slight
	Squinting	June 10	Slight
	Stain, Abdomen, Yellow	June 10	Moderate
	Dehydration	June 15	Slight
86C00240	Inactive	June 10-12	Marked
	Tremors	June 10-12	Moderate
	Squinting	June 10-13	Moderate
	Ataxia	June 10-12	Slight
	Rough Coat	June 10,13-16	Slight
	Stain, Perianal, Yellow	June 13-16	Slight
	Hyperactive	June 14,15	Slight

APPENDIX E (cont.): INDIVIDUAL ANIMAL HISTORIES

MALE: 3980 mg/kg DIGL-RP

Animal Number	Clinical Signs	Dates Observed (1986)	Severity
86C00171	Hunched Posture	June 19,21-24	Marked
	Inactive	June 19,21-23	Marked
	Ataxia	June 19,21	Moderate
	Tremors	June 19	Slight
	Incr. Startle Reflex	June 19	Slight
	Rough Coat	June 24	Slight
86C00179	Hunched Posture	June 19,20	Moderate
	Inactive	June 19,20	Moderate
	Tremors	June 19,20	Moderate
	Incr. Startle Reflex	June 19,20	Slight
	Twitching	June 19	Slight
	Squinting	June 20	Moderate
	Moribund	June 21,22	N/A
	Death	June 23	3.9 days
86C00197	Hunched Posture	June 19	Moderate
	Hyperactive	June 19	Moderate
	Twitching	June 19	Moderate
	Ataxia	June 19	Moderate
	Tremors	June 19,20	Moderate
	Inactive	June 19,20	Marked
	Death	June 21	1.9 days
86C00210	Rough Coat	June 19,20	Moderate
	Hunched Posture	June 19,20	Moderate
	Squinting	June 19	Slight
	Stain, Perianal, Yellow	June 20	Slight
86C00216	Hunched Posture	June 19,20	Moderate
	Ataxia	June 19,20	Moderate
	Hyperactive	June 19	Slight
	Squinting	June 19	Slight
	Rough Coat	June 19	Slight
	Tremors	June 19,20	Moderate
86C00218	Hunched Posture	June 19	Slight
	Inactive	June 19	Slight
	Squinting	June 19	Slight
	Incr. Startle Reflex	June 19	Slight
	Rough Coat	June 19	Slight
	Ataxia	June 20	Moderate
	Tremors	June 20	Slight

APPENDIX E (cont.): INDIVIDUAL ANIMAL HISTORIES

MALE: 3980 mg/kg DIGL-RP (cont.)

Animal Number	Clinical Signs	Dates Observed (1986)	Severity
86C00228	Hunched Posture	June 19	Moderate
	Tremors	June 19,20	Marked
	Inactive	June 19,20	Marked
	Ataxia	June 19,20	Marked
	Incr. Startle Reflex	June 19,20	Moderate
	Squinting	June 19,20	Marked
	Twitching	June 19	Slight
	Stain, Abdomen, Yellow	June 20	Slight
	Death	June 21	1.9 days
86C00229	Hunched Posture	June 19	Moderate
86C00232	Hunched Posture	June 19,20	Moderate
	Ataxia	June 19,20	Moderate
	Inactive	June 19,20	Marked
	Tremors	June 19,20	Moderate
	Twitching	June 19,20	Moderate
	Depr. Righting Reflex	June 19	Moderate
	Squinting	June 19,20	Marked
	Rough Coat	June 19	Slight
	Prostrate	June 21	N/A
	Death	June 22	2.9 days
86C00235	Ataxia	June 19,20	Moderate
	Tremors	June 19-21	Slight
	Hunched Posture	June 19,20	Slight
	Inactive	June 19-21	Moderate
	Prostrate	June 21	N/A
	Death	June 22	2.9 days

APPENDIX E (cont.): INDIVIDUAL ANIMAL HISTORIES

MALE: 4122 mg/kg DIGL-RP

Animal Number	Clinical Signs	Dates Observed (1986)	Severity
86C00168	Hunched Posture	June 10,11	Moderate
	Ataxia	June 10,11	Slight
	Inactive	June 10,11	Moderate
	Rough Coat	June 10,11	Slight
	Stain, Abdomen, Yellow	June 10,11	Slight
	Death	June 12	1.9 days
86C00169	Hunched Posture	June 10-12	Marked
	Tremors	June 10,11	Marked
	Squinting	June 10-12	Marked
	Inactive	June 10,11	Marked
	Prostrate	June 12	N/A
	Pallor	June 12	Marked
	Death	June 12	2.2 days
86C00188	Hunched Posture	June 10,11	Moderate
	Squinting	June 10,11	Marked
	Ataxia	June 10,11	Moderate
	Tremors	June 10,11	Moderate
	Inactive	June 10,11	Marked
	Rough Coat	June 10,11	Moderate
	Stain, Abdomen, Yellow	June 10,11	Slight
	Death	June 12	1.9 days
86C00191	Ataxia	June 10	Slight
	Tremors	June 10	Slight
	Hunched Posture	June 10	Moderate
	Squinting	June 10,11	Slight
	Inactive	June 11	Moderate
86C00200	Ataxia	June 10-12	Slight
	Hunched Posture	June 10-14	Moderate
	Squinting	June 10-12	Moderate
	Tremors	June 10,11	Marked
	Inactive	June 12	Moderate
	Rough Coat	June 14-16	Slight

APPENDIX E (cont.): INDIVIDUAL ANIMAL HISTORIES

MALE: 4122 mg/kg DIGL-RP (cont.)

Animal Number	Clinical Signs	Dates Observed (1986)	Severity
86000207	Inactive	June 10	Slight
86000209	Inactive	June 10-12	Moderate
	Hunched Posture	June 10-12	Moderate
	Tremors	June 10	Slight
	Stain, Abdomen, Yellow	June 10,11,13	Slight
	Rough Coat	June 14-17	Moderate
86000227	Inactive	June 10,11	Marked
	Hunched Posture	June 10,11	Slight
	Squinting	June 10,11	Moderate
	Rough Coat	June 10,11, 13,15,16	Moderate
	Stain, Abdomen, Yellow	June 10,11, 13,14	Slight
	Tremors	June 12,13	Slight
	Stain, Perianal, Yellow	June 15,16	Slight
86000241	Inactive	June 10,11	Marked
	Squinting	June 10,11	Moderate
	Hunched Posture	June 10	Slight
	Stain, Abdomen, Yellow	June 10,11	Slight
	Ataxia	June 11	Moderate
	Death	June 12	1.9 days
86000244	Misdosed	N/A	N/A

APPENDIX E (cont.): INDIVIDUAL ANIMAL HISTORIES

MALE: 4558 mg/kg DIGL-RP

Animal Number	Clinical Signs	Dates Observed (1986)	Severity
86C00166	Inactive	June 10,11	Marked
	Ataxia	June 10	Slight
	Hunched Posture	June 10	Slight
	Rough Coat	June 10,11	Slight
	Tremors	June 11	Moderate
	Squinting	June 11	Moderate
	Death	June 12	1.9 days
86C00170	Hunched Posture	June 10	Slight
	Ataxia	June 10-12	Marked
	Inactive	June 10-12	Marked
	Tremors	June 10,11	Slight
	Squinting	June 12	Marked
	Death	June 13	2.9 days
86C00178	Tremors	June 10,11	Marked
	Ataxia	June 10,11	Moderate
	Tachypnea	June 10	Slight
	Hunched Posture	June 10	Slight
	Depr. Righting Reflex	June 10	Moderate
	Squinting	June 11	Marked
	Death	June 12	1.9 days
86C00181	Inactive	June 10,11	Marked
	Depr. Righting Reflex	June 10	Slight
	Hunched Posture	June 10	Moderate
	Squinting	June 10,11	Marked
	Tremors	June 10,11	Moderate
	Prostrate	June 10,11	N/A
	Death	June 12	1.9 days
86C00189	Tremors	June 10,11	Marked
	Hunched Posture	June 10	Moderate
	Squinting	June 10,11	Marked
	Ataxia	June 10	Moderate
	Inactive	June 10	Moderate
	Prostrate	June 10,11	
	Death	June 12	1.9 days

APPENDIX E (cont.): INDIVIDUAL ANIMAL HISTORIES

MALE: 4558 mg/kg DIGL-RP (cont.)

Animal Number	Clinical Signs	Dates Observed (1986)	Severity
86C00193	Tremors	June 10,11	Moderate
	Ataxia	June 10,11	Moderate
	Hunched Posture	June 10	Slight
	Squinting	June 10,11	Marked
	Inactive	June 10,11	Marked
	Stain, Abdomen, Yellow	June 10,11	Slight
	Death	June 12	1.9 days
86C00214	Hunched Posture	June 10	Marked
	Squinting	June 10-12	Marked
	Inactive	June 10,11	Marked
	Tremors	June 10,11	Moderate
	Stain, Abdomen, Yellow	June 10-12	Slight
	Prostrate	June 12	N/A
	Death	June 12	2.2 days
86C00222	Inactive	June 10,11	Moderate
	Hunched Posture	June 10	Slight
	Tremors	June 10	Slight
	Squinting	June 11	Slight
	Ataxia	June 11	Slight
86C00231	Inactive	June 10,11	Marked
	Hunched Posture	June 10-14	Moderate
	Tremors	June 10,11	Marked
	Squinting	June 10,11	Marked
86C00236	Ataxia	June 10,11	Slight
	Squinting	June 10-14	Marked
	Tremors	June 10,11	Moderate
	Hunched Posture	June 10,11	Moderate
	Inactive	June 12	Slight
	Rough Coat	June 13	Slight

APPENDIX E (cont.): INDIVIDUAL ANIMAL HISTORIES

MALE: Vehicle Control

Animal Number	Clinical Signs	Dates Observed (1986)	Severity
86C00004	Normal	N/A	N/A
86C00010	Normal	N/A	N/A
86C00029	Normal	N/A	N/A
86C00044	Normal	N/A	N/A
86C00083	Hyperactive Hunched Posture	March 22-26 March 24,25	Moderate Slight

APPENDIX E (cont.): INDIVIDUAL ANIMAL HISTORIES

FEMALE: 2196 mg/kg DIGL-RP

Animal Number	Clinical Signs	Dates Observed (1986)	Severity
86C00085	Normal	N/A	N/A
86C00106	Irritable	March 19	Slight
	Hyperactive	March 19-26	Slight
86C00107	Tremors	March 19	Moderate
	Inactive	March 19	Slight
	Hunched Posture	March 19	Slight
	Rough Coat	March 20,21	Slight
86C00115	Tremors	March 23	Slight
86C00117	Removed From Study	N/A	N/A
86C00137	Hyperactive	March 19,22-30	Moderate
	Incr. Respiratory Rate	March 25,26	Slight
86C00141	Alopecia	April 2	Slight

APPENDIX E (cont.): INDIVIDUAL ANIMAL HISTORIES

FEMALE: 3092 mg/kg DIGL-RP

Animal Number	Clinical Signs	Dates Observed (1986)	Severity
86C00086	Inactive	March 26,27	Moderate
	Tremors	March 26,27	Slight
	Hunched Posture	March 26,27	Slight
86C00087	Tremors	March 26	Slight
	Hunched Posture	March 26,27	Slight
	Hyperactive	March 27	Slight
86C00089	Tremors	March 26-29	Marked
	Hunched Posture	March 26,29	Moderate
	Incr. Startle Reflex	March 26	Slight
	Twitching	March 26	Moderate
	Prostrate	March 27,28	N/A
	Rough Coat	March 30	Slight
86C00092	Tremors	March 26,27	Slight
	Hunched Posture	March 26	Moderate
	Depr. Grasping Reflex	March 26	Slight
86C00097	Tremors	March 26,27	Slight
	Rough Coat	March 26	Slight
	Depr. Grasping Reflex	March 26	Moderate
	Hunched Posture	March 26-28	Moderate
	Hyperactive	March 31-April 9	Moderate
86C00135	Tremors	March 26,27	Marked
	Hunched Posture	March 26	Moderate
	Depr. Righting Reflex	March 26	Moderate
	Prostrate	March 27	N/A
	Death	March 27	24.0 h
86C00143	Hyperactive	March 26	Slight
	Hunched Posture	March 26	Slight

APPENDIX E (cont.): INDIVIDUAL ANIMAL HISTORIES

FEMALE: 3858 mg/kg DIGL-RP

Animal Number	Clinical Signs	Dates Observed (1986)	Severity
86C00101	Tremors	April 9,10	Moderate
	Inactive	April 9	Slight
	Depr. Righting Reflex	April 9	Slight
	Squinting	April 9,10	Moderate
	Depr. Grasping Reflex	April 9	Moderate
	Prostrate	April 10	N/A
	Death	April 11	1.9 days
86C00126	Tremors	April 9-11	Moderate
	Inactive	April 9,11	Marked
	Depr. Righting Reflex	April 9	Slight
	Hunched Posture	April 9,10	Moderate
	Squinting	April 9,10	Moderate
	Ataxia	April 9-11	Marked
	Rough Coat	April 10	Moderate
	Death	April 11	2.1 days
86C00146	Tremors	April 9-11	Moderate
	Inactive	April 9-11	Marked
	Hunched Posture	April 9	Slight
	Squinting	April 9	Moderate
	Ataxia	April 10,11	Marked
	Writhing	April 10	Marked
	Death	April 12	2.8 days
86C00149	Inactive	April 9-11	Marked
	Tremors	April 9-11	Moderate
	Depr. Righting Reflex	April 9	Slight
	Hunched Posture	April 9	Moderate
	Squinting	April 9-11	Slight
	Incr. Startle Reflex	April 9	Slight
	Ataxia	April 10,11	Marked
	Death	April 11	2.0 days

APPENDIX E (cont.): INDIVIDUAL ANIMAL HISTORIES

FEMALE: 3858 mg/kg DIGL-RP (cont.)

Animal Number	Clinical Signs	Dates Observed (1986)	Severity
86C00152	Tremors	April 9,10	Moderate
	Depr. Grasping Reflex	April 9	Moderate
	Ataxia	April 9,10	Moderate
	Inactive	April 9,10	Marked
	Hunched Posture	April 9	Slight
	Squinting	April 10	Moderate
	Death	April 11	1.9 days
86C00156	Tremors	April 9	Moderate
	Hunched Posture	April 9,10	Moderate
86C00162	Inactive	April 9-11	Marked
	Tremors	April 9-11	Marked
	Squinting	April 9	Moderate
	Incr. Startle Reflex	April 9	Slight
	Hunched Posture	April 9,10	Slight
	Stain, Perianal, Yellow	April 10	Slight
	Ataxia	April 11	Marked
	Death	April 11	2.2 days

APPENDIX E (cont.): INDIVIDUAL ANIMAL HISTORIES

FEMALE: 4454 mg/kg DIGL-RP

Animal Number	Clinical Signs	Dates Observed (1986)	Severity
86C00128	Inactive	April 2,3	Moderate
	Tremors	April 2,3	Slight
	Hunched Posture	April 2,3	Slight
	Death	April 4	2.9 days
86C00131	Inactive	April 2,3	Moderate
	Tremors	April 2,3	Marked
	Squinting	April 2,3	Slight
	Depr. Righting Reflex	April 2,3	Marked
	Depr. Grasping Reflex	April 2,3	Marked
	Prostrate	April 2,3	N/A
	Death	April 4	1.9 days
86C00142	Inactive	April 2	Moderate
	Tremors	April 2,3	Marked
	Prostrate	April 2,3	N/A
	Depr. Grasping Reflex	April 2,3	Marked
	Depr. Righting Reflex	April 2,3	Marked
	Squinting	April 2,3	Moderate
	Hunched Posture	April 2,3	Slight
	Death	April 3	28.0 h
86C00144	Hunched Posture	April 2	Moderate
	Inactive	April 2	Moderate
	Tremors	April 2,3	Marked
	Twitching	April 2,3	Moderate
	Depr. Grasping Reflex	April 2,3	Marked
	Squinting	April 2,3	Marked
	Prostrate	April 3	N/A
	Death	April 4	1.9 days
86C00159	Hunched Posture	April 2,3	Slight
	Inactive	April 2,3	Moderate
	Tremors	April 2,3	Moderate
	Prostrate	April 2,3	N/A
	Squinting	April 2,3	Moderate
	Depr. Grasping Reflex	April 2,3	Marked
	Death	April 4	1.9 days

APPENDIX E (cont.): INDIVIDUAL ANIMAL HISTORIES

FEMALE: 4454 mg/kg DIGL-RP (cont.)

Animal Number	Clinical Signs	Dates Observed (1986)	Severity
86C00161	Inactive	April 2	Moderate
	Tremors	April 2,3	Moderate
	Hunched Posture	April 2,3	Moderate
	Depr. Grasping Reflex	April 2,3	Marked
	Squinting	April 2,3	Marked
	Prostrate	April 2,3	N/A
	Death	April 4	1.9 days
86C00164	Inactive	April 2,3	Marked
	Tremors	April 2,3	Marked
	Incr. Startle Reflex	April 2,3	Slight
	Squinting	April 2,3	Marked
	Depr. Righting Reflex	April 2,3	Moderate
	Prostrate	April 2-11	N/A
	Opisthotonus	April 3	Slight
	Death	April 4	1.9 days

APPENDIX E (cont.): INDIVIDUAL ANIMAL HISTORIES

FEMALE: Vehicle Controls

Animal Number	Clinical Signs	Dates Observed (1986)	Severity
86000091	Normal	N/A	N/A
86000100	Hyperactive	March 24,25,27	Slight
86000116	Normal	N/A	N/A
86000130	Normal	N/A	N/A
86000150	Rough Coat	March 20-23	Slight
	Infection, Cervical Tag	March 26,27,31 April 1,2	N/A

Appendix F: INDIVIDUAL BODY WEIGHTS IN GRAMS

Males: 3516 mg/kg DIGL-RP

Animal No.	Receipt	Dosing	Day 7	Termination Day 14
86C00176	33	38	38	40
86C00177	29	34	Dead	---
86C00182	30	36	37	40
86C00183	30	33	33	33
86C00187	31	37	38	39
86C00190	34	38	38	41
86C00196	30	37	35	37
86C00202	30	34	33	35
86C00204	33	39	39	41
86C00205	30	37	36	37
86C00208	30	37	34	35
86C00213	27	31	30	31
86C00215	30	33	33	36
86C00220	29	30	31	34
86C00224	29	35	33	35
86C00226	30	30	33	34
86C00233	33	38	36	38
86C00237	28	31	32	32
86C00239	33	36	35	36
86C00240	30	32	29	34
Mean	30.4	34.8	34.4	36.2
Standard Deviation	1.8	2.9	2.9	3.0
Std. Error of Mean	0.4	0.6	0.7	0.7

Appendix F (cont.): INDIVIDUAL BODY WEIGHTS IN GRAMS

Males: 3980 mg/kg DIGL-RP

Animal No.	Receipt	Dosing	Day 7	Termination Day 14
[illegible]171	27	32	27	31
[illegible]179	31	40	Dead	---
[illegible]197	28	34	Dead	---
[illegible]0210	30	32	33	37
[illegible]0216	27	29	28	31
[illegible]0218	31	34	35	36
[illegible]0228	29	27	Dead	---
[illegible]0229	28	27	28	29
[illegible]0232	26	33	Dead	---
[illegible]0235	29	34	Dead	---
Mean	28.6	32.2	30.2	32.8
Standard Deviation	1.7	3.9	3.6	3.5
Std. Error of Mean	0.5	1.2	1.6	1.6

Appendix F (cont.): INDIVIDUAL BODY WEIGHTS IN GRAMS

Males: 4122 mg/kg DIGL-RP

Animal No.	Receipt	Dosing	Day 7	Terminal Day 14
86C00168	32	35	Dead	---
86C00169	28	32	Dead	---
86C00188	29	33	Dead	---
86C00191	33	36	38	39
86C00200	30	31	28	36
86C00207	29	37	36	38
86C00209	29	33	33	38
86C00227	30	35	33	37
86C00241	33	34	Dead	---
Mean	30.2	34.0	33.6	37.6
Standard Deviation	2.0	1.9	3.1	1.1
Std. Error of Mean	0.7	0.6	1.7	0.5

Appendix F (cont.): INDIVIDUAL BODY WEIGHTS IN GRAMS

Males: 4558 mg/kg DIGL-RP

Animal No.	Receipt	Dosing	Day 7	Termination Day 14
[illegible]166	25	31	Dead	---
[illegible]170	29	34	Dead	---
[illegible]178	28	31	Dead	---
[illegible]181	28	31	Dead	---
[illegible]189	30	33	Dead	---
[illegible]193	31	33	Dead	---
[illegible]214	27	32	Dead	---
[illegible]222	30	35	32	35
[illegible]231	32	34	33	35
[illegible]236	32	38	35	41
Mean	29.2	33.2	33.5	37.0
Standard Deviation	2.2	2.2	1.5	3.5
Std. Error of Mean	0.7	0.7	0.7	2.0

Appendix F (cont.): INDIVIDUAL BODY WEIGHTS IN GRAMS

Males: Vehicle Control

Animal No.	Receipt	Dosing	Day 7	Termination Day 14
86C00004	24	31	33	34
86C00010	26	30	32	33
86C00029	26	32	36	36
86C00044	23	30	34	36
86C00083	25	30	31	32
Mean	24.8	30.6	33.2	34.2
Standard Deviation	1.3	0.9	1.9	1.8
Std. Error of Mean	0.6	0.4	0.9	0.8

Appendix F (cont.): INDIVIDUAL BODY WEIGHTS IN GRAMS

Females: 2196 mg/kg DIGL-RP

Animal No.	Receipt	Dosing	Day 7	Termination Day 14
[illegible]085	25	27	29	31
[illegible]106	26	29	30	32
[illegible]107	28	31	31	31
[illegible]115	25	30	31	32
[illegible]137	29	32	33	33
[illegible]141	27	31	31	32
Mean	26.7	30.0	30.8	31.8
Standard Deviation	1.6	1.8	1.3	0.8
Std. Error of Mean	0.7	0.7	0.5	0.3

Appendix F (cont.): INDIVIDUAL BODY WEIGHTS IN GRAMS

Females: 3092 mg/kg DIGL-RP

Animal No.	Receipt	Dosing	Day 7	Termination Day 14
86C00086	27	30	34	33
86C00087	28	34	34	33
86C00089	25	30	30	31
86C00092	25	30	29	31
86C00097	24	32	32	32
86C00135	28	31	Dead	---
86C00143	23	31	31	30
Mean	25.7	31.1	31.7	31.7
Standard Deviation	2.0	1.5	2.1	1.2
Std. Error of Mean	0.7	0.6	0.8	0.5

Appendix F (cont.): INDIVIDUAL BODY WEIGHTS IN GRAMS

Females: 3858 mg/kg DIGL-RP

Animal No.	Receipt	Dosing	Day 7	Termination Day 14
86C00101	22	27	Dead	---
86C00126	22	32	Dead	---
86C00146	26	30	Dead	---
86C00149	26	32	Dead	---
86C00152	26	29	Dead	---
86C00156	30	36	36	36
86C00162	25	30	Dead	---
Mean	25.3	30.9	36.0	36.0
Standard Deviation	2.8	2.9	N/A	N/A
Std. Error of Mean	1.0	1.1	N/A	N/A

Appendix F (cont.): INDIVIDUAL BODY WEIGHTS IN GRAMS

Females: 4454 mg/kg DIGL-RP

Animal No.	Receipt	Dosing	Day 7	Termination Day 14
86C00128	25	33	Dead	---
86C00131	29	30	Dead	---
86C00142	24	30	Dead	---
86C00144	26	30	Dead	---
86C00159	26	34	Dead	---
86C00161	26	30	Dead	---
86C00164	23	31	Dead	---
Mean	25.6	31.1	---	---
Standard Deviation	1.9	1.7	---	---
Std. Error of Mean	0.7	0.6	---	---

Appendix F (cont.): INDIVIDUAL BODY WEIGHTS IN GRAMS

Females: Vehicle Controls

Animal No.	Receipt	Dosing	Day 7	Termination Day 14
86C00091	26	30	33	33
86C00100	23	27	29	29
86C00116	24	28	28	29
86C00130	28	31	31	32
86C00150	24	29	29	31
Mean	25.0	29.0	30.0	30.8
Standard Deviation	2.0	1.6	2.0	1.8
Std. Error of Mean	0.9	0.7	0.9	0.8

Appendix G: PATHOLOGY REPORT

GLP Study #85022
Principal Investigator: MAJ Brown

I. INTRODUCTION

Study: Oral LD50/DIGL-RP Solid Propellant
Animal: Mouse (ICR)/albino
Reference: SOP OP-STX-36

II. SUMMARY OF PROCEDURES

Euthanasia: Sodium Pentobarbital
Fixative: 10% buffered formalin
Histopathology: Routine
Clinical Lab: None

III. GROSS FINDINGS

Males - 3516 mg/kg DIGL-RP
(Live animals indicated by '*'/Histopathology by '$')

ACCESSION NUMBER	ANIMAL ID NUMBER	DOSE-DEATH INTERVAL	OBSERVATIONS
* 39773	86C00176	14 Days	Not Remarkable (NR)
39762	86C00177	2 Days	Test compound in stomach
* 39774	86C00182	14 Days	NR
* 39810	86C00183	14 Days	NR--ID tag missing
* 39811	86C00187	14 Days	NR
* 39812	86C00190	14 Days	NR
* 39813	86C00196	14 Days	NR
* 39777	86C00202	14 Days	NR
* 39814	86C00204	14 Days	NR
* 39815	86C00205	14 Days	NR
* 39779	86C00208	14 Days	NR
* 39781	86C00213	14 Days	NR
* 39817	86C00215	14 Days	NR
* 39782	86C00220	14 Days	NR
* 39784	86C00224	14 Days	NR
* 39820	86C00226	14 Days	NR
* 39787	86C00233	14 Days	NR
* 39822	86C00237	14 Days	NR
* 39789	86C00239	14 Days	NR
* 39790	86C00240	14 Days	NR

Appendix G (cont.): PATHOLOGY REPORT

Males - 3980 mg/kg DIGL-RP
(Live animals indicated by '*'/Histopathology by '$')

ACCESSION NUMBER	ANIMAL ID NUMBER	DOSE-DEATH INTERVAL	OBSERVATIONS
* 39809	86C00171	14 Days	NR
39765	86C00179	4 Days	Hepatic pallor, mild Gastric mucosa, petechia, mild Small intestine, hemorrhage, diffuse, mild
39760	86C00197	2 Days	Test compound in stomach Intestine, postmorten autolysis, mild
* 39816	86C00210	14 Days	NR
* 39818	86C00216	14 Days	NR
* 39819	86C00218	14 Days	NR
39761	86C00228	2 Days	Test compound in stomach Postmortem autolysis, moderate
* 39821	86C00229	14 Days	NR
39763	86C00232	3 Days	Test compound in stomach Small intestinal hemorrhage, segmental, mild Pulmonary congestion Postmortem autolysis, moderate
39764	86C00235	3 Days	Hepatic pallor, mild Test compound in stomach Gastric mucosa petechiae with hemorrhage Ileal hemorrhage, diffuse, mild

Males - 4122 mg/kg DIGL-RP

39745	86C00168	2 Days	NR
$ 39753	86C00169	3 Days	Hepatic pallor, moderate
39748	86C00188	2 Days	Hepatic pallor, mild
* 39775	86C00191	14 Days	NR
* 39776	86C00200	14 Days	NR--ID tag missing
* 39778	86C00207	14 Days	NR
* 39780	86C00209	14 Days	NR
* 39785	86C00227	14 Days	NR
39751	86C00241	2 Days	NR--postmortem autolysis, moderate

Appendix G (cont.): PATHOLOGY REPORT

Males - 4558 mg/kg DIGL-RP (cont.)
(Live animals indicated by '*'/Histopathology by '$')

ACCESSION NUMBER	ANIMAL ID NUMBER	DOSE-DEATH INTERVAL	OBSERVATIONS
39744	86C00166	2 Days	Hepatic pallor, mild Pulmonary congestion
39756	86C00170	3 Days	Hepatic postmortem changes
39746	86C00178	2 Days	Hepatic pallor, mild
39747	86C00181	2 Days	NR
39749	86C00189	2 Days	NR
39750	86C00193	2 Days	NR-postmortem autolysis, mild
39754	86C00214	3 Days	Hepatic pallor, mild Postmortem autolysis, mild
* 39783	86C00222	14 Days	NR
* 39786	86C00231	14 Days	NR
* 39788	86C00236	14 Days	NR

Males - Vehicle Controls

* 39453	86C00004	14 Days	Not remarkable (NR)
* 39452	86C00010	14 Days	NR
* 39449	86C00029	14 Days	NR
* 39451	86C00044	14 Days	NR
* 39450	86C00083	14 Days	NR

Females - Vehicle Controls

* 39455	86C00091	14 Days	NR
* 39456	86C00100	14 Days	NR
* 39460	86C00116	14 Days	NR
* 39461	86C00130	14 Days	NR
* 39464	86C00162	14 Days	NR

Females - 2196 mg/kg DIGL-RP

* 39454	86C00085	14 Days	NR
* 39457	86C00106	14 Days	NR
* 39458	86C00107	14 Days	NR
* 39459	86C00115	14 Days	NR
* 39462	86C00137	14 Days	NR
* 39463	86C00141	14 Days	NR

Appendix G (cont.): PATHOLOGY REPORT

Females - 3092 mg/kg DIGL-RP
(Live animals indicated by '*'/Histopathology by '$')

ACCESSION NUMBER	ANIMAL ID NUMBER	DOSE-DEATH INTERVAL	OBSERVATIONS
* 39502	86C00086	14 Days	NR
* 39503	86C00087	14 Days	NR
* 39504	86C00089	14 Days	NR
* 39505	86C00092	14 Days	NR
* 39506	86C00097	14 Days	NR
$ 39383	86C00135	1 Day	Pulmonary congestion, diffuse, mild
* [illegible]	86C00143	14 Days	NR

Females - 3858 mg/kg DIGL-RP

39510	86C00101	2 Days	NR
39512	86C00126	3 Days	NR
39516	86C00146	3 Days	Hepatic pallor, mild
39513	86C00149	2 Days	NR
39511	86C00152	2 Days	NR
* 39515	86C00156	14 Days	NR
39514	86C00162	3 Days	NR

Females - 4454 mg/kg DIGL-RP

39479	86C00128	3 Days	NR
39480	86C00131	2 Days	NR
$ 39472	86C00142	2 Days	Pulmonary congestion, mild ID tag missing
39481	86C00144	2 Days	NR
39482	86C00159	2 Days	NR
39483	86C00161	2 Days	NR
39484	86C00164	2 Days	NR

IV. SUMMARY OF GROSS FINDINGS: Gross findings consisted of two groups of changes. The first group included hepatic pallor, pulmonary congestion, gastrointestinal hemorrhages and the presence of test compound in the stomach while the second group of changes included a variety of autolytic and other postmortem changes.

Appendix G (cont.): PATHOLOGY REPORT

V. MICROSCOPIC FINDINGS

39753	LIVER - 1. Vacuolar change, diffuse, mild 2. Congestion, diffuse, mild
39383	LUNG - Congestion, diffuse, mild SMALL INTESTINE - NR STOMACH - NR LARGE INTESTINE - NR
39472	LUNG - Congestion, diffuse, minimal

VI. SUMMARY OF MICROSCOPIC FINDINGS: The microscopic appearance of this hepatic vacuolar change is consistent with fat and probably accounted for the hepatic pallor noted grossly. Pulmonary congestion, also noted grossly, was confirmed microscopically. Hepatic congestion, on the other hand, was not noted grossly.

VII. CONCLUSIONS

Gross and microscopic changes were noted only in animals submitted dead and represented lesions commonly seen with the agonal process sometimes associated with death and postmortem changes, with the exception of gastrointestinal hemorrhage, which is typical for this class of compounds. In this project, such hemorrhage was noteworthy in the high dose males.

HARRY L. WALKER, DVM
CPT, VC
Division of Pathology

MICHAEL V. SLAYTER, DVM
MAJ, VC
Chief, Division of Pathology

14 August 1986

dbj

Distribution List

Commander
US Army Biomedical Research and Development Laboratory (12)
ATTN: SGRD-UBZ-C
Fort Detrick, Frederick, MD 21701-5010

Defense Technical Information Center (DTIC) (2)
ATTN: DTIC-DLA
Cameron Station
Alexandria, VA 22304-6145

US Army Medical Research and Development Command (2)
ATTN: SGRD-RMI-S
Fort Detrick, Frederick, MD 21701-5012

Commandant
Academy of Health Sciences, US Army
ATTN: AHS-CDM
Fort Sam Houston, TX 78234

Chief
USAEHA Regional Division, West
Fitzsimmons AMC
Aurora, CO 80045

Chief
USAEHA Regional Division, North
Fort George G. Meade, MD 20755

Chief
USAEHA Regional Division, South
Bldg. 180
Fort McPherson, GA 30330

Commander
USA Health Services Command
ATTN: HSPA-P
Fort Sam Houston, TX 78234

Commander US Army Materiel Command
ATTN: AMSCG
5001 Eisenhower Avenue
Alexandria, VA 22333

Commander
US Army Environmental Hygiene Agency
ATTN: Librarian, HSDH-AD-L
Aberdeen Proving Ground, MD 21010

Dean
School of Medicine
Uniformed Services University of the Health Sciences
4301 Jones Bridge Road
Bethesda, MD 20014

Commander
US Army Materiel Command
ATTN: AMCEN-A
5001 Eisenhower Avenue
Alexandria, VA 22333

HQDA
ATTN: DASG-PSP-E
Falls Church, VA 22041-3258

HQDA
ATTN: DAEN-RDM
20 Massachusetts, NW
Washington, D.C. 20314

CDR, US Army Toxic and Hazardous Material Agency
ATTN: DRXTH/ES
Aberdeen Proving Ground, MD 21010

Commandant
Academy of Health Sciences
United States Army
ATTN: Chief, Environmental Quality Branch
Preventive Medicine Division (HSHA-IPM)
Fort Sam Houston, TX 78234